A HISTOCHEMICAL STUDY OF INFLAMMATORY PROLIFERATION OF EPITHELIUM

Ou Bao-syan

Laboratory for the Study of Carcinogenic Agents (Head, Corresponding Member AMN SSSR, L. M. Shabad) Institute of Experimental and Clinical Oncology (Director, Active Member AMN SSSR, N. N. Blokhin)* AMN, SSSR, Moscow
(Presented by Active Member AMN SSSR, A. D. Timofeevskii)
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A study of the relationships between epithelium and the connective tissue in deep inflammatory epithelial growths is extremely important in connection with the mechanism of the invasive growth of normal and malignant cells. V. G. Garshin [3] has made a detailed study of the pathogenesis of these outgrowths, and has concluded that their formation and regression is related to the condition of the connective tissue. A. A. Zavarzin [4] has pointed out that the epithelium grows into immature connective tissue consisting of fibroblasts; the more slowly the tissue matures, the longer the infiltration of epithelium continues. N. N. Anichkov and V. G. Garshin [1], and A. A. Braun [2] have shown that during wound healing, the epithelium grows over granulation tissue of a certain degree of maturity. A. A. Zavarzin [4] thought that deep epithelial growth took place when there was a break in the basement membrane. According to Z. S. Katsnel'son [5], the basement membrane is formed when the relationship between the epithelium and connective tissue has become stabilized. However, S. K. Tezekbaev [6] observed buried outgrowths of epithelium while the basal membrane was intact.

To decide the problem of the relationships between epithelium and connective tissue, important evidence can be obtained from an analysis of the histochemical changes observed during inflammatory outgrowths. However, practically no work on these lines has been done.

The object of the present investigation has been to study inflammatory epithelial outgrowths.

EXPERIMENTAL METHOD

Inflammatory outgrowths of epithelium were induced in rabbit ear skin by injecting a saturated solution of scharlach red in sunflower oil into the subcutaneous connective tissue. The experiments were performed on 9 rabbits. They were killed 3, 5, 7, 11, 15, 25, 40, 50, and 60 days after the injection of the stimulant. Pieces of normal ear skin and of the tissue surrounding the solution of the dye were fixed in formol and in Carnoy's fluid, embedded in paraffin, stained in hematoxylin-eosin, in picrofuchsine, or impregnated with silver by Gomori's method. We used the following histochemical reactions: Brachet's ribonucleic acid method, Feulgen's desoxyribonucleic acid, the tetrazolium coupling reaction which reveals various protein groups, Barnet and Zeligman's reaction with DDD which reveals sulphydril groups, the reaction of Barnet, Tsou, and Zeligman for the carboxyl groups of proteins; acid mucopolysaccharides were identified with toluidine blue and alcyanin blue and control preparations were treated with hyaluronidase; polysaccharides containing 1, 2-glycol groups were revealed by the periodate-Schiff reagent (Schiff reaction), and control preparations were treated with amylase.

EXPERIMENTAL RESULTS

Scharlach red induced characteristic changes of the epithelium and subcutaneous connective tissue of the ear, which passed through several stages: 1) hyperplasia of the epidermis and of the hair follicles (on the third day after injecting the dye); this hyperplasia was apparently associated with the remote influence of the source of inflammation, because at this stage in the immediate neighborhood of the epithelium, there was no inflammatory infiltration; 2) the formation of primary epithelial outgrowths, which infiltrated into the inflammatory zone (on the 5th day); the connective tissue around the outgrowths became looser, and then on the seventh day young fibroblasts appeared; 3) the epithelization of the cyst containing the injected solution (on the 7th day) and the formation of atypical and secondary outgrowths (on the 11th-15th days); around them there were many young fibroblasts arranged at random;

* Yu. M. Vasil'ev, Senior Scientific Assistant and Doctor of Medical Sciences, took part in carrying out the work.

Fig. 1. Hyperplasia of the epidermis. Tetrazolium coupling reaction. Formation of an epithelial outgrowth in the skin of the ear of a rabbit 5 days after injecting scharlach red. The intensity of the reaction increases from the basal to the superficial layer of the hyperplasic epithelium. The most marked reaction is given by the central cornified portion. Magnification 590.

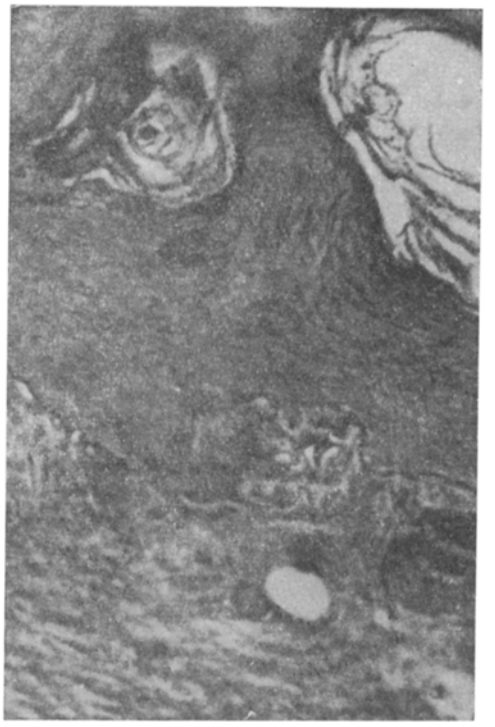

Fig. 3. Basement membrane giving a positive reaction around atypical outgrowths of the epithelium, 11 days after injecting scharlach red. Reaction for carboxyl groups. Magnification 590.

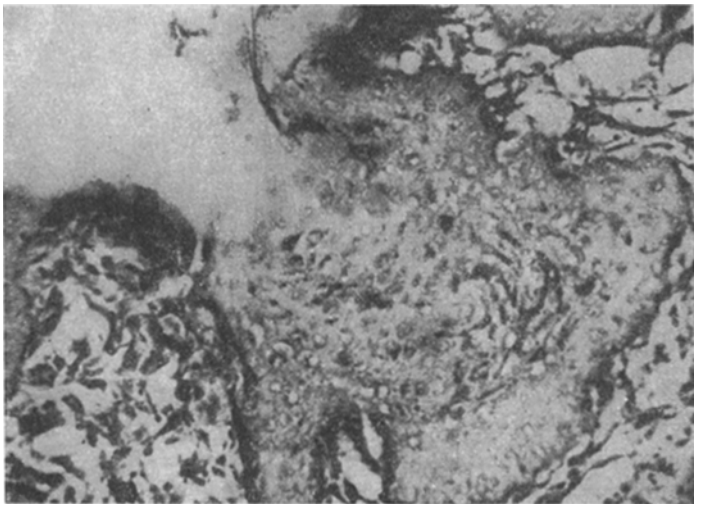

Fig. 2. Schiff-positive granules in cytoplasm of cells of a protuberant atypical outgrowth of the epithelium 7 days after injecting scharlach red, and Schiff-positive reaction of the basal membrane.

4) the transformation of the atypical epithelial outgrowths of the primary and secondary growths into "resting epithelial islets" (on the 15th- 25th day) and, (on the 25th- 60th day) into epidermoid cysts surrounded by maturing connective tissue, but itself subject to slow regression, though persisting to some extent until the 40th- 60th day.

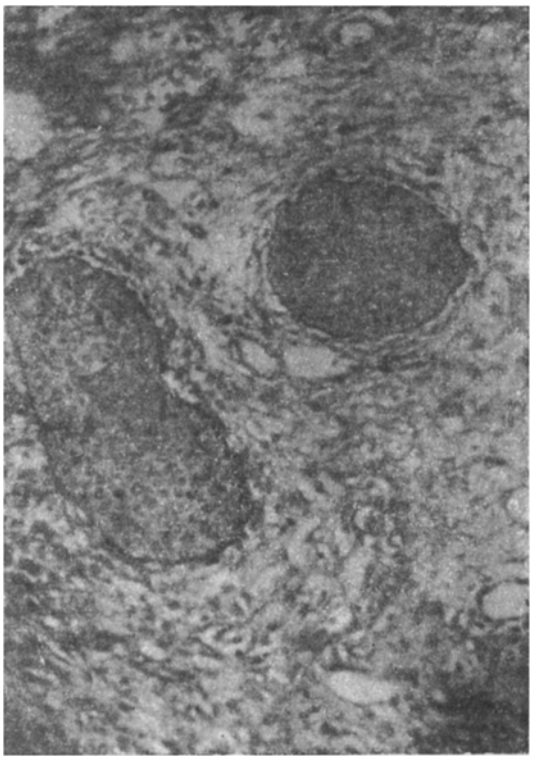

Fig. 4. Islets of an epithelium in the connective tissue, 14 days after injecting scharlach red. Silver impregnation by Gomori's method. The basement membrane around the islets is well preserved. Magnification 590.

At all stages of the epithelial outgrowth, there was a visible increase in the intensity of the epithelial cytoplasmic RNA reaction. The strongest RNA reaction was in the cells of the atypical outgrowths (on the 7th-11th day). These outgrowths were surrounded by a large number of young fibroblasts, whose cytoplasm also gave an intense RNA reaction. Later (on the 15th-25th day), as the connective tissue matured, the outgrowths became converted into "resting islets" and the intensity of the reaction of their cytoplasm for RNA was reduced; at the same time there was a reduction also in the amount of RNA in the cytoplasm surrounding these groups of fibroblasts. There is therefore a certain correlation between the intensity of the RNA reaction in the epithelium and in the connective tissue cells. Possibly the proliferation of the epithelium in the atypical outgrowths is due to the influence of the RNA-containing components of the surrounding fibroblasts.

To obtain precise information on the RNA content of the cells during the inflammatory outgrowth of the epithelium, quantitative photometric studies are required.

During differentiation (cornification) of the epithelial epidermal cells, a protein which gives the tetrazolium coupling reaction and which contains aminoacylcarboxylic and sulphydril groups [8] accumulates in the cytoplasm. Therefore the intensity of the reaction for these groups increases from the basal to the superficial layer of the normal hyperplastic epithelium and of the epithelium of the epidermoid cysts, while in the outgrowths and in the large compartments the most marked reactions are given by the central cornified portions (Fig. 1). In the atypical outgrowths of the epithelium and in the small islets, these reactions are weak, evidently because of the low degree of differentiation of the cells of these outgrowths.

A chromotropic mucopolysaccharide, which can be broken down by testicular hyaluronidase, appeared in the ground substance of the connective tissue at the early stages of the formation of collagen, in regions remote from the epithelial outgrowths, but the tissue stained orthochromatically around the atypical epithelial outgrowths. At the later stages, this mucopolysaccharide spread diffusely both close to and far from the epithelial outgrowths. Later still (on the 25th - 60th day) the chromotropic mucopolysaccharide could not be found in those parts of the tissue where the formation of collagen fibers had already finished; at these stages a mucopolysaccharide appeared near certain of the "resting epithelial islets." Apparently the development of collagen in the connective tissue around the epithelial islets begins and ends later than elsewhere in the connective tissue. These results indicate that the invasive epithelial growth is not, as Sylven [9] and certain other authors have supposed, associated with the accumulation of chromotropic mucopolysaccharides in the connective tissue surrounding the epithelium.

Granules containing glycogen are not present in the epithelial cells of the normal epidermis, but appear in the cytoplasm of the hyperplastic epithelium; the number of such granules increases in the cytoplasm of the atypical outgrowths. The number of such granules are especially numerous in the early stages of cornification. In the cells of the "resting islets" and of the epidermoid cysts, the amount of glycogen is negligible. The cytoplasm of the young fibroblasts surrounding the atypical outgrowths contains a Schiff-positive component which differs from glycogen by resisting treatment with amylase.

The basement membrane of the normal epithelium is argyrophil, and gives a Schiff positive reaction; however, it gives negative reactions for tetrazolium and sulphydril groups, and for carboxyl groups of proteins. During

hyperplasia of the epithelium and the formation of the initial outgrowth, the structure of the basement membranes is well preserved (Fig. 2); only in certain regions is there some partial disintegration of these membranes. In some parts, the atypical outgrowths have no basement membrane. However, in may other regions, the edge of the outgrowing epithelium is surrounded by well defined basement membranes, which, as well as being argyrophil and giving a Schiff-positive reaction, also begin to give a positive reaction for functional protein groups (Fig. 3). At the later stages, around the "resting islets" basement membranes are formed, but in their histochemical reactions they differ from the basement membranes of the active epithelium. These membranes remain argyrophil (Fig. 4), but in most cases they lose their Schiff-positive component, and the component which gives a positive tetrazolium coupling reaction and a reaction for carboxyl and sulphydril groups. Changes of the basement membranes in inflammatory outgrowths of the cutaneous epithelium of rabbits have much in common with those found in the invasive growth of the epithelium of the mammary glands of mice during pregnancy [7].

SUMMARY

The amounts of ribonucleic acid and glycogen-containing granules increased in the cytoplasm of epithelial cells during inflammatory proliferation of the cutaneous epithelium of the rabbit ear. Differentiation of the hyperplastic epithelial cells was accompanied by an increased intensity of the tetrazolium coupling reaction, as well as by increased reactions for sulphydril and carboxylic groups. The invasive growth of the epithelium was associated with destruction of the basement membrane, or with the accumulation of chromotropic mucopolysaccharides near the epithelial outgrowths.

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